Action potentials in maize sieve tubes change phloem translocation

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Abstract

The inhibition of phloem translocation caused by electric- and cold-shock was studied by macro- and microautoradiography in mature leaves of maize ( Zea mays L.). In addition, both types of stimulation triggered action potentials with amplitudes of more than 50 mV which were transmitted without diminution in sieve tubes with velocities of 3-5 cm s\(^{-1}\). By utilizing X-ray microanalysis ion concentrations of sieve tubes were measured in non-stimulated and stimulated leaves. It was shown that potassium and chloride diminished about 3-fold after stimulation while the amount of cytoplasmic calcium may have increased. These displacements lead to the conclusion that calcium influx as well as potassium and chloride efflux are involved in the propagation of action potentials. A possible relationship between electric signalling and the reduction of phloem translocation is discussed.

Key words: Action potential, electric and cooling stimuli, energy-dispersive X-ray microanalysis, ion shifts, phloem translocation.

Introduction

With regard to the interruption of phloem translocation it is well-known that abrupt temperature drops (Pickard et al., 1978; Minchin and Thorpe, 1983) or electric shocks (Pickard and Minchin, 1990, 1992) can produce such effects. From the latter reports it is known that the inhibition depends on a threshold electric field strength at 0.5 V mm\(^{-1}\) and that higher currents, which produce higher electric fields in the tissue, are associated with inhibitions of greater duration. To explain such phenomena, Pickard and Minchin (1992) provided a conceptual model in terms of voltage-gated ion channels in the phloem, which may open in response to suitable polarization of the symplasm and, secondly, pass a biochemical messenger which initiates the inhibition of phloem translocation. The present paper explores the topic of how electric shock-induced changes of ion distributions become physiologically transduced into interruption of phloem transport. Therefore, the displacements of various ions in sieve tubes of maize were observed during electric stimulation. In addition, phloem translocation was monitored by macro- and microautoradiography and the membrane potential of sieve tubes was measured by using severed aphid stylets as first described by Wright and Fisher (1981). The results shown here confirm the model of voltage-gated ion channels in sieve tubes and present evidence of an involvement of action potentials in the interruption of phloem translocation.

Materials and methods

Plant material

Maize ( Zea mays L.) was grown in climate rooms under a light intensity of 400 Jm\(^{-2}\)s\(^{-1}\) provided by mercury halide lamps in a 14/10 h light/dark period at a temperature of 24 °C and 70% RH.

Arrangement of experiments

When the plants had about 10 visible leaves, they were predarkened for 48 h. From three plants identical in height the blades of the 5th leaf from the top were simultaneously sealed gas-tight in a plexiglas box (Fig. 1). The plants were illuminated with 400 Jm\(^{-2}\)s\(^{-1}\) from mercury halide lamps (Osram, Berlin, FRG) and the blades inside the box were exposed to \(^{14}\)CO\(_2\) (2.9 MBq \(^{14}\)C) for 30 min. During this time the middle plant (2), which served as control, was never touched and thus remained unstimulated. In contrast, both other plants were stimulated once per minute during the translocation period of 30 min. The leaf blade of the plant behind (1) was stimulated electrically by a silver wire electrode, applied to the leaf surface 1 cm basipetally to the exposed box. The stimuli with a strength...
of 10 V and a duration of 4 s were delivered each minute using a pulse generator (A.M.P.I., Jerusalem, Israel), an adjustable voltage source. From the third plant the sealed leaf blade (3) was stimulated with ice water (4°C) once per minute, applied to the distal area of the leaf 5 cm acropetally to the plexiglas box.

After a period of 30 min all three leaves were quickly frozen in dry ice and freeze-dried for macroautoradiography. Microautoradiographs were prepared according to Fritz (1980). Small sections (1–2 mm in diameter) of the leaves were quickly frozen in isopentane which was pre-cooled by liquid nitrogen. After freeze-drying and embedding in Spurr’s resin medium (Spurr, 1969) sections were cut with a Reichert Ultracut E microtome and fixed with 0.1 N NaOH and 5% H2O2, which later improved the staining with 0.05% toluidine blue, pH 7.0. The sections were coated with liquified photographic emulsion (Ilford L4), exposed for 6 weeks and developed in D-19 A/S developer (Sanderson, 1981).

For X-ray microanalysis the embedded plant material was cut dry with Teflon-coated glass knives; sections were coated with a thin layer of carbon in a vacuum evaporator. X-ray microanalysis was carried out with a Philips 420 transmission electron microscope equipped with an energy-dispersive X-ray microanalytical device (EDAX 9100). The measurements were made at a magnification of 6350 x with 120 keV, the diameter of the incident electron beam was 200–250 nm. The spectra are plotted as mean values attained from histologically identical locations. Quantification was done according to the method described by Hall (1971) and Cliff and Lorimer (1975).

Measurement of electric potential differences in the phloem

Before the measurements started, the plants were placed in the Faraday cage for 1 d and the stimulating electrodes were applied to the surface of a mature leaf. An aphid cage with 10–15 aphids (Rhopalosiphum padi) was applied to the leaf basipetally of the site of stimulation. The aphids were allowed to settle overnight. On the following day, an aphid feeding on the lower side of the leaf which produced honeydew was severed from its stylet by using a laser beam generator (Beck, Neu-Isenburg, FRG) connected to a Zeiss microscope. When the stylet stump exuded sieve-tube sap, all other aphids were brushed away and the leaf was kept at about 90% relative humidity. Then the exudate droplet on the stylet was brought into contact with the tip of a microelectrode by using a Leitz micromanipulator (Fig. 2). The glass microelectrode had a tip diameter of approximately 1 μm and was fabricated from microcapillaries (WPI, Sarasota, Florida, USA) on a vertical electrode puller (Getra, München, FRG) and back-filled with 100 mol m⁻³ KCl. The microelectrode was clamped in a Ag-AgCl pellet holder (WPI) and connected to a microelectrode preamplifier (input impedance >10¹² ohms) to which a WPI-amplifier (Model 750) was attached. The resistance for an electric current inside the stylet is relatively low (around 10⁶ ohms according to Wright and Fisher, 1981) compared to the high input impedance of the electric equipment used. The reference electrode was brought into contact with the nutrient solution (15 mol m⁻³) of the root system. This sort of measurement entails recording across a number of equivalent resistances and does not reflect the membrane electrical properties usually measured in isolated cells.

For comparison, a second microelectrode was impaled into the leaf mesophyll. By using a long-working distance objective the position of the microelectrode was checked to be in the mesophyll. Action potentials were generated by ice water as well as electrically by using the same pulse generator as described in the transport section. The outputs were displayed on a chart recorder.

Results

Action potentials in sieve tubes

As generally known from observations on many plants, electric activity in plants can be checked by stimulating the plant with ice water. In our experiments the tip of the microelectrode was brought into contact with the exudate droplet on the aphid stylet (Fig. 2) while the reference electrode was immersed into the nutrient solution of the root system. The resting potential of the measured cells ranged from −140 mV to −155 mV during six experiments with different plants. This is similar to sieve tube potentials of Mimosa (Fromm, 1991) and barley leaves (Fromm and Eschrich, 1989). When the leaf tip was stimulated with ice water, the microelectrode recorded a basipetally propagating action potential with a depolarizing amplitude of 80 mV in the sieve tubes (Fig. 3, upper scheme). The velocity of signal transmission was 3–5 cm s⁻¹.

During electrical stimulation of the leaf tip, a stimulus artifact, which does not reflect the electrical activity of the plant, was observed (Fig. 3, lower scheme). However, it slightly hyperpolarized the phloem potential in most experiments. Following electrical stimulation a propagating action potential was measured in the sieve tube system with a velocity of 5 cm s⁻¹. Stimuli shorter than 2 s or
Fig. 2. After the aphid was severed from its mouthparts by shooting with a microscope laser, the stylet stump exuded sieve tube sap to which the tip of a microelectrode was attached by using a micromanipulator.

Fig. 3. Measurements of intracellular potentials in sieve tubes of maize via severed aphid stylets. Stimulation by ice water (above) and electric shock (below) evoked action potentials which were propagated with a velocity of 3–5 cm s\(^{-1}\) in a basipetal direction.

lower than 3 V did not induce an action potential, regardless of intensity or duration of the other parameter. With regard to refractory periods, we found that for 50 s after the action potential has passed it is impossible to elicit a second one, however high the stimulus strength. When the microelectrode was impaled into the leaf mesophyll, the resting potential ranged from \(-110\) to \(-130\) mV during the experiments with six plants. Following stimulation of the leaf tip, potential changes similar to the sieve tube action potential were measured. In contrast to the sieve tube system, the transmission velocity was in the range of 0.2 to 2 cm s\(^{-1}\). However, we are not sure of the direction in a 2 or 3 dimensional anisotropic material. It could also be that the transmission pathway is down the phloem and then very slowly across the mesophyll.

**The effect of stimulation on phloem translocation**

Macroautoradiographs of the source leaves exposed to \(^{14}\)CO\(_2\) showed clear differences between the stimulated leaves on the left and right side as compared to the non-stimulated leaf in the middle (Fig. 4). In the latter, which served as control, the \(^{14}\)C-labelled photoassimilates extended in the expected normal distribution from the exposed middle area of the source leaf towards the base. No label was imported into the tip of the mature leaf.

When the leaf of the plant was stimulated electrically 2 cm from the \(^{14}\)C-exposed box (Fig. 4; left leaf, open arrow) the pattern of labelling disclosed an interruption...
of phloem translocation. In contrast, veins on the right side to the stimulated region of this leaf still transported assimilates towards the base. However, they are not so densely labelled as the veins of the control leaf. When the leaf of the third plant was simultaneously stimulated with ice water at the leaf tip acropetally to the $^{14}$C-exposed box (Fig. 4; right leaf, black arrow), again, phloem translocation decreased sharply in all veins of the leaf. This inhibition did not occur when water at a temperature of 25 °C was applied to the leaf surface. So far, experiments showed that each stimulation (electrically or cooling) which produced propagating action potentials in sieve tubes, also caused a reduction of phloem transport. Surprisingly, the reduction of translocation produced by an abrupt temperature drop is not restricted to the area where it is applied to the leaf, but also influences assimilate transport 10–20 cm basipetally. The microautoradiographic evaluation of the leaf area basipetally to the exposed box showed that in non-stimulated leaves $^{14}$C-label was concentrated in the phloem (Fig. 5). However, microautoradiographs of the leaf area stimulated electrically disclosed $^{14}$C-labelling inside and outside the vascular tissues (Fig. 6). The phloem cells are only slightly labelled and only some photoassimilates are unloaded from the phloem into the surrounding tissue. This shows that the treatment interrupts phloem translocation within the sieve tubes and does not cause extended phloem unloading in the stimulated leaf region.

**Ion concentrations in non-stimulated and stimulated sieve tubes**

In Fig. 7, concentrations of potassium, chloride and calcium are shown for the sieve element cytoplasm in both unstimulated leaves and electrically-stimulated leaf areas. The graphs show that $K^+$ and $Cl^-$ have concentrations of 170 to 180 mM in the unstimulated leaves. In contrast, the $Ca^{2+}$ level is below 2 mM. In sieve tubes of the stimulated leaf region, $K^+$ decreased to 30 mM and $Cl^-$ to 50 mM while $Ca^{2+}$ may have increased slightly. In the sieve element apoplast of these leaf areas $K^+$ and $Cl^-$ increased during stimulation. Thus, there is strong evidence that stimulation affects voltage-gated ion channels. The ionic mechanism of excitation in plants is based on the observation that chloride carried the inward current.

![Fig. 5. Microautoradiograph of a part of the vascular bundle of the region basipetal to the exposed box of the non-stimulated leaf. Label concentrates at sites of phloem strands (P). V, vessel; S, sclerenchyma.](image1)

![Fig. 6. Microautoradiograph of a vascular bundle of the electrically stimulated leaf area. The phloem (P) is not densely labelled; instead some label has spread into vessels (V) and bundle-sheath cells (BS).](image2)
Action potentials in phloem

Concentration of potassium ($K^+$), chloride ($Cl^-$), and calcium ($Ca^{2+}$) in the sieve element cytoplasm of unstimulated and electrically-stimulated maize leaves. Data are mean values ± s.e. of 10 measurements from two plants.

Fig. 7

Discussion

From studies of numerous plant species it is suggested that action potentials play a major role in inter- and intracellular communication and for regulation of physiological processes at the molecular and the organism level (Davies, 1987). Action potentials have not only been examined in plants possessing motor activity but also in Characean algal cells (Findlay, 1961; Gaffey and Mullins, 1958; Oda, 1976; Shimmen and Tazawa, 1980), Acetabularia (Gradmann, 1976), the liverwort Conocephalum conicum (Dziubinska et al., 1983) and in higher plants like Helianthus annuus (Zawadzki et al., 1991) and Salix viminalis (Fromm and Spanwick, 1993; Fromm and Eschrich, 1993). Evidence for a link between electric signalling and biochemical response was given recently by Wildon et al. (1992). After wounding tomato plants, a close relationship was observed between induced electric signals and the activation of proteinase inhibitor genes.

With regard to the function of the phloem, it has been shown that action potentials propagating in sieve tubes of Mimosa trigger phloem unloading in the pulvini (Fromm, 1991).

In the present study cold-shock as well as electrical stimulation caused action potentials in maize, propagating away from the site of stimulation. Stimulation with ice water has been reported to induce propagating action potentials in a number of plant species, including Biophyllum (Sibaoka, 1973) as well as pumpkin and tomato (Van Sambeek and Pickard, 1976). Since Woodley et al. (1976) have found that localized chilling temporarily stops or reduces translocation in sunflowers for 10–15 min and that this reduction in translocation corresponds closely with electrical changes measured along the stem, the idea of a relationship between action potentials and the cold-shock-induced inhibition of phloem transport came up. Faucher et al. (1982) found that maize leaves which were quickly cooled to $1^\circ C$ showed a complete inhibition of translocation, in contrast to slowly cooled leaves which were scarcely affected. They assumed that some sort of bioelectric variation may be involved in the cold-shock-induced inhibition of translocation.

Obviously, there exists a relationship between cold-shock-induced action potentials and interruption of phloem transport because both of them depend on rapid temperature drops. When the temperature is decreased slowly neither phloem transport is inhibited nor action potentials are elicited. Minchin and Thorpe (1983) found that rapid temperature drops of only $2.5^\circ C$ caused a brief abeyance of phloem transport in Ipomea purpurea, Ipomea alba, Phaseolus vulgaris, and Nymphoides geminata, which was not observed when the temperature was decreased slowly.

In the present study in maize it is shown that rapid cold-shock treatments cause sieve elements to trigger action potentials, most probably by activating voltage-dependent ion channels in the sieve element plasmalemma. Simultaneously, phloem translocation is reduced sharply, visible more than 15 cm from the area of stimulation by macroautoradiography.

In addition to cooling, the electroshock-induced inhibition of phloem translocation was investigated in detail by Pickard and Minchin (1992) in the bean stem. They suggested that the electroshock-inhibition is the result of ordinary voltage-gated ion channels in phloem cells.
which, in response to suitable polarization of the symplasm open and pass a biochemical messenger which initiates the observed interruption of translocation. In maize, action potentials are elicited by electrical stimulation and the K⁺ and Cl⁻ concentrations of the sieve elements decreased sharply while Ca²⁺ may have increased. From these ion displacements, it is assumed that Cl⁻ also carries the inward and K⁺ the outward current in the maize action potential. However, the amount of KCl required to sustain action potentials is too small to explain the ionic shifts in the mM-range. Since Ca²⁺ is present at lower concentrations than K⁺ and Cl⁻, its role during excitation might involve stimulation, as was already proposed for the Characeae action potential (Lunevsky et al., 1983). Evidence for a link between electric signalling and the interruption of phloem translocation is given by the decrease in symplastic K⁺ and Cl⁻ concentration. According to Shiina and Tazawa (1986) who investigated the effects of action potentials on the elongation growth in the stem of Luffa cylindrica, K⁺ and Cl⁻ efflux from stimulated cells into the apoplastic space may reduce cell turgor and cause growth retardation. In maize, the reduction of K⁺ and Cl⁻ in the sieve tube symplasm during excitation might also reduce cell turgor and trigger water efflux out of sieve tubes, because K⁺ and Cl⁻ moved into the apoplast. Assuming that assimilate transport in sieve tubes probably requires the passive movement of water as the transport medium, a reduction in turgor could cause a reduction in phloem translocation as shown in stimulated maize leaves. On the other hand the reduced translocation rate could be explained by a decrease of the phloem loading process which occurs along the whole leaf blade and is expected to depend on the sieve tube membrane potential as well as the potassium concentration. Thus, the repeated irritation of the membrane potential which is propagated throughout the whole plant might primarily block apoplastic phloem loading. However, the controlling points in this transport system are the subject of further investigations.

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